

Int. J. Aquat. Biol. (2015) 3(6): 425-432
E-ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com
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Original Article

Toxic effects of Cadmium on antioxidant defense systems and lipid peroxidation in *Acipenser persicus* (Borodin, 1897)

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Abstract: Cadmium is considered as a common residue in water and sediments which could readily enter aquatic organisms. The aim of this study was to evaluate the time- and concentration-dependent changes in antioxidant enzymes (SOD and CAT) activities as well as concentration of MDA as a bi-product of lipid peroxidation in the liver of Persian sturgeon, *Acipenser persicus*, following CdCl₂ exposure at sub-lethal concentrations for 14 days. Based on the results, activity of SOD and CAT showed a significant increase in the fish exposed to different concentrations of CdCl₂ up to the day 7, and then their activity decreased in the fish of all treatments on the 14th day. In all treatments, MDA content significantly increased after exposure at first day until the end of the experiment. The levels of SOD, CAT and MDA followed a concentration-dependent manner and its increase was higher in 800 µg l⁻¹ than those of 200 µg l⁻¹. The results suggested that antioxidant enzymes could be used as an effective index to monitor ecotoxicological changes.

Article history:

Received 6 September 2015

Accepted 1 December 2015

Available online 25 December 2015

Keywords:

Oxidative stress
Superoxide dismutase
Catalase
Malondialdehyde
Persian sturgeon

Introduction

Cadmium is considered as a highly toxic metal pollutant which easily enters the environment via industrial and agricultural activities such as mining, ore refining, electroplating processes, chemical fertilizers and pesticides (Stohs and Baghchi, 1995; Duribe et al., 2007). It is a serious threat to the environment due to its persistent nature, long distance transport and toxicity to aquatic organisms (Huang et al., 2005). Even at low concentrations, this metal may accumulate in aquatic animal's body and resulting in several toxic effects, including tissue damage, physiological and biochemical alterations, respiratory changes, and ultimately death (Reynders et al., 2006; Oner et al., 2008; Banaee et al., 2015). The intensity and duration of these responses are influenced by several factors such as concentration of the toxicant, time of exposure and the fish species (Dabas et al., 2012).

Alterations in biochemical levels are usually the first detectable responses to environmental perturbation

which can provide information on sub-lethal cellular effects of stress factors in a particular species and have potential to be applied as sensitive biomarkers in field studies to monitor fish health (Kim et al., 2008). Among the most commonly used biochemical biomarkers, those related to oxidative stress are considered to have an important role, being frequently used both in environmental monitoring and laboratory assays (Pandey et al., 2003). In fact, the metabolism of xenobiotics is a two-phase process. The reactions of the first phase include oxidation, reduction and hydrolysis in which the most important ones are oxidation enzymes involved in metabolism of the majority of xenobiotics. In the second phase of the reactions, endogenous enzymatic and non-enzymatic antioxidants converse the reactive oxygen species (ROS) to harmless types and also protect and restore normal cellular metabolisms and functions as well (Ballesteros et al., 2009). Elevated levels of ROS cause oxidative damage in biomolecules such as lipids, proteins and

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DNA (Livingstone, 2003; Sevcikova et al., 2011). The key enzymes for detoxifying ROS in all organisms are superoxide dismutase, glutathion-s-transferase and catalase (Lonidis and Yu, 1995; Wang et al., 2008; Ballesteros et al., 2009; Dazy et al. 2009; Rastgoo and Alemzadeh, 2011). Estimation of lipid peroxidation has been also found in various studies to have predictive importance as a biomarker of oxidative stress (Lackner et al., 1998).

Persian sturgeon, *Acipenser persicus*, has been listed as an endangered species, existing in the Caspian Sea, the largest continental water body on the earth containing more than 85% of the world's sturgeon population (Pourang et al., 2005). Besides overfishing, contamination by industrial, domestic effluents and other anthropological activities (Kaplin, 1995; Lenhardt et al., 2006) result in declining its population to non-economic levels over the past decades.

Induction of oxidative stress has been reported in fish exposed to pollutants (Dorts et al., 2009; Almeida et al., 2009; Sharma et al., 2013; Sharma et al., 2014). Considering these effects, studies on the effect of Cd on *Acipenser* species are limited to some studies including LC₅₀ estimates (Mirzaee et al., 2003), stress indices (Glucose, Cortisol, ALT, and AST) (Zahedi et al., 2013), cytotoxicity (Shariati et al., 2011) and genotoxicity (Safari et al., 2014). Therefore, this study was designed to evaluate the time- and concentration-dependent changes in antioxidant enzymes (SOD and CAT) activities as well as the concentration of MDA as a bi-product of lipid peroxidation in liver of *A. persicus* following CdCl₂ exposure at sub lethal concentrations for 14 days.

Materials and Methods

Fingerlings of the Persian sturgeon (3-5 g) were obtained from Shahid Marjani Breeding and Rearing Center (Golestan Province, Iran). They were acclimated to the experimental conditions for two weeks. Then the fish were randomly distributed into 9 tanks of 300 L at a density of 30 fish per tank and submitted to 200 and 800 µg l⁻¹ CdCl₂ for 14 days

(LC₅₀ was previously determined as 4000 µg l⁻¹, Shariati et al., 2011). Three tanks (each for one replication) were considered for each treatment. During the exposure, the fish were fed with *Artemia* biomass twice a day and the water was continuously monitored for temperature, dissolved oxygen, pH and conductivity (mean ± SD., T ~ 24 ± 1°C; DO ~ 7 ± 0.2 mg L⁻¹; pH ~ 7.6 ± 0.2; 1412 ± 167.9 Ms/cm). **Oxidative stress analysis:** Nine fish per treatment were rapidly anesthetized by clove powder (0.5 g L⁻¹) solution; their liver were removed and then immediately deep-frozen in liquid nitrogen and stored at -80°C for further analysis.

Sample preparation: The sampled livers were homogenized (1:5) in ice-cold 50 mM phosphate buffer pH 7.5 containing protease inhibitor cocktail (sigma p2714). The homogenate was centrifuged at 10000 g for 10 min at 4°C and supernatant was kept at -20°C for antioxidant enzyme activities assays. Protein content was assayed based on Lowery et al. (1951).

Activity of antioxidant enzymes

Superoxide dismutase (SOD): The SOD activity was measured according to its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) as described by Dhindsa et al. (1981). The activity of SOD was expressed as unit per milligram protein.

Catalase (CAT): Activity of CAT was assayed using spectrophotometric method by determining H₂O₂ decomposition in time at 240 nm based on Aebi (1984). The enzyme activity was expressed as unit per milligram protein.

Malondialdehyde (MDA): LPO which results in production of malondialdehyde (MDA) and consequently to free radicals, was assessed by thiobarbituric acid reactive substances (TBARS) (Fatima et al., 2000). MDA reacts with thiobarbituric acid (TBA) and the product is read by spectrophotometric method at 535 nm. The homogenate was added at the ratio of 1:1 (v:v) to 5% trichloroacetic acid (TCA), and incubated on ice for 15 min. The solution was then mixed at the ratio of 2:1 with 0.67% TBA, and centrifuged at 2200×g at

Table 1. Alternation in SOD (U/mg) activity in the liver of *Acipenser persicus* exposed to 200 and 800 $\mu\text{g l}^{-1}$ of Cd for 14 days.

Exposure day/ Concentration	1	4	7	14
control	22.63 \pm 0.3 ^a _A	22.4 \pm 0.28 ^c _A	22.4 \pm 0.24 ^c _A	22.4 \pm 0.1 ^b _A
200 $\mu\text{g l}^{-1}$	22.7 \pm 0.12 ^a _C	23.45 \pm 0.24 ^b _{BC}	31.26 \pm 0.6 ^a _A	27.3 \pm 0.4 ^a _{AB}
800 $\mu\text{g l}^{-1}$	22.96 \pm 0.2 ^a _B	27.65 \pm 0.2 ^a _A	28.11 \pm 0.3 ^a _A	27.76 \pm 0.0.3 ^a _A

The results are expressed as means with standard deviation (n=9). Different superscript and subscript letters denote significant ($P<0.05$) difference in each column (a-c) and each row (A-D), respectively.

Table 2. Alternation in CAT (U/mg) activity in the liver of *Acipenser persicus* exposed to 200 and 800 $\mu\text{g l}^{-1}$ of Cd for 14 days.

Exposure day/ Concentration	1	4	7	14
control	2.76 \pm 0.1 ^a _A	2.9 \pm 0.1 ^b _A	3 \pm 0.1 ^b _A	2.88 \pm 0.16 ^c _A
200 $\mu\text{g l}^{-1}$	2.9 \pm 0.4 ^a _C	3.27 \pm 0.15 ^b _C	7.8 \pm 0.3 ^a _A	5.8 \pm 0.28 ^a _B
800 $\mu\text{g l}^{-1}$	3 \pm 0.3 ^a _C	5.8 \pm 0.3 ^a _{AB}	6.72 \pm 0.4 ^a _A	4.92 \pm 0.22 ^b _B

The results are expressed as means with standard deviation (n=9). Different superscript and subscript letters denote significant ($P<0.05$) difference in each column (a-c) and each row (A-D), respectively.

Table 3. Alternation in MDA (nmol g^{-1} tissue) in the liver of *Acipenser persicus* exposed to 200 and 800 $\mu\text{g l}^{-1}$ of endosulfan for 14 days.

Exposure day/ Concentration	1	4	7	14
control	6.5 \pm 0.6 ^a _A	6.2 \pm 0.9 ^b _A	6.8 \pm 0.2 ^c _A	6.4 \pm 0.7 ^b _A
200 $\mu\text{g l}^{-1}$	6.5 \pm 0.5 ^a _D	9 \pm 0.34 ^b _C	10.5 \pm 0.25 ^a _B	13.5 \pm 0.66 ^a _A
800 $\mu\text{g l}^{-1}$	6.7 \pm 0.4 ^a _D	12.2 \pm 0.4 ^a _C	14 \pm 0.9 ^b _B	17 \pm 0.59 ^a _A

The results are expressed as means with standard deviation (n=9). Different superscript and subscript letters denote significant ($P<0.05$) difference in each column (a-c) and each row (A-D), respectively.

4°C for 10 min. The whole supernatant was boiled for 10 min, and then refreshed at room temperature before recording the absorbency. A calibration curve with increasing MDA concentrations allowed the calculation of LPO expressed as nmol g^{-1} per tissue.

Statistical analysis: For each index, the data were tested for normality and homogeneity. Normalized data passed Levens test for homogeneity of variance. Statistics data were subjected to one way ANOVA ($\alpha = 0.05$). Comparisons within each analysis day and within a treatment at different sampling days were made by Duncan's test. Data were reported as mean \pm standard deviation. The SPSS software, version 16, was utilized for data analysis (SPSS, Richmond, Virginia, USA).

Results

After exposure to the selected concentrations of cadmium chloride, the toxic stress on fish was manifested in the form of restlessness and jerky and erratic swimming movements. Instantaneous secretion of excessive mucus all over the body

surface of the exposed fish also was notable especially in higher concentration. The exposed fish rejected feeding in the earlier stages (up to the 4th day) of exposure, then, they started to consume the food (with hesitation) gradually and resumed feeding nearly as normal. Throughout the experiment, no significant macroscopic behavioral changes were observed in the control group. No death was recorded either in the control or experimental group during the experiment.

Enzymes Assay: Activity of both antioxidant enzymes (SOD and CAT) in liver of Cd (200 and 800 $\mu\text{g l}^{-1}$) exposed fish (*A. persicus*) generally increased compared to that of the control group until the 7th day of exposure ($P<0.05$) followed by non-significant decrease on the day 14. The activity of enzymes significantly increased in the fish treated with 800 more than 200 $\mu\text{g l}^{-1}$ CdCl₂ (Tables 1 and 2).

Lipid peroxidation: In the exposed fish, TBARS levels increased compared to that of the control group. In both experimental concentrations, MDA significantly increased at first day ($P<0.05$). MDA

content followed a concentration-dependent trend and grew in the fish treated with 800 more than 200 $\mu\text{g l}^{-1}$ CdCl_2 (Table 3).

Discussion

Application of pollutant biomarkers has been the subject of various studies, especially because of the fact that distinct kinds of pollutants may interfere with animal physiology and behavioral processes, which is also of ecological importance (Scott and Solman, 2004). Cadmium dose not generate ROS directly, but can alter antioxidant enzymes, especially SOD, CAT and GSH and it is able to displace copper and iron in various proteins, and then freeing these metals to participate in the Fenton reaction (Ercal et al., 2001; Remeo et al., 2000; Sevcikova et al., 2011). Based on the results, Cd exposure increased SOD and CAT in the liver of Persian sturgeon in comparison with that of the control indicating that the antioxidant system response to cadmium stress. A significant increase in SOD and CAT enzymes until the 7th day of exposure could be attributed to superoxide radical accumulation. Similar results were reported in *Oreochromis mossambicus* (Basha and Rani, 2003), *Channa punctatus* (Dabas et al., 2012), and *O. niloticus* (Saglam et al., 2014) which were attributed to superoxide radical accumulation, de-novo synthesis of enzymatic proteins and inducing expression of genes encoding SOD and CAT to detoxify ROS (Rastgoo and Alemzadeh, 2011).

The depressed catalytic activity of CAT and SOD on the 14th day of exposure may be linked to the binding of Cd to -SH groups of these enzymes. The present findings are in agreement with the results obtained in cadmium exposed *Danio rario* (Ghazie et al., 2013) and *Arius arius* (Mani et al., 2014) in which it was reported that binding of non-essential heavy metals to the active center of enzyme resulted in overproduction of H_2O_2 that consequently caused decline in antioxidant enzymes activity. Falling trend of antioxidant enzymes activity was attributed to the flux of superoxide radicals by Filek et al. (2008) and Modesto and Martinez (2010). These bi-phasic

responses have been reported in several studies (Garcia Sampaio et al., 2008; Saglam et al., 2014). The antioxidant enzyme responses to pollutant could be species-specific in addition to the factors such as dosage and exposure duration (Almeida et al., 2009; Dorts et al., 2009; Jia et al., 2011; Wang et al., 2013). In this study, the activity of antioxidant enzymes increased in higher concentration treatments and followed a concentration-dependent trend. A contrary result was observed in *Lutjanus argentimaculatus* after exposure to high levels of Cd compared to low dosages and suggested that low dosages of Cd might enhance the protective mechanism, while high dosages lead gradually to cytotoxicity and inhibit the antioxidant enzymes (Wang et al., 2013).

Lipid peroxidation (LPO) is also one of the key manifestations of oxidative damage induced by various compounds, including metals (Ercal et al., 2001; Dabas et al., 2012). In this study, MDA increased up to the 14th day of exposure to Cd. The determined values of LPO were in agreement with those reported for other fish species (Pandey et al., 2008). Cd produces inhibitory effects on mitochondria electron transport (Belyaeva et al., 2012), and as a result, the respiratory chain becomes highly reduced and the electrons are transferred directly to available oxygen, leading to an enhancement in ROS formation (Miyamoto et al., 2003), which cause peroxidative damage in liver. Increasing MDA has been reported after exposure to Cd in *O. niloticus* (Almedia et al., 2009), *C. Puntatus* (Dabas et al., 2012), *Clarias gariepinus* (Asagba et al., 2008), and *L. argentimaculatus* (Wang et al., 2013).

In this study, TBARS levels followed a concentration-dependent manner. In addition, the elevated LPO levels are supported by the results reported in *C. gariepinus* (Asagba et al., 2008) and *C. Puntatus* (Dabas et al., 2012). Contrary to these results, Wang et al. (2013) showed higher content of MDA at low concentrations of Cd due to inhabitation of the antioxidant enzymes.

In conclusion, exposure to sub-lethal dosage of

CdCl₂ showed a biphasic trend in antioxidant enzymes. Antioxidant enzymes showed a rise until the 7th day and MDA levels increased up to the end of experiment. The present results suggested that antioxidant enzymes could be used as an effective index to monitor ecotoxicological changes.

Acknowledgements

The author is grateful to Gorgan University of Agricultural Sciences and Natural Resources for providing facilities to accomplish this study.

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چکیده فارسی

تأثیر کادمیوم بر سیستم دفاع آنتی اکسیدانسی و پراکسیداسیون چربی‌ها در تاس‌ماهی ایرانی (*Acipenser persicus* (Borodin, 1897))

رقیه صفری

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چکیده:

کادمیوم یکی از پسماندهای معمول در محیط‌های آبی و رسوبات است که به آسانی وارد بدن آبزیان می‌شود. هدف این مطالعه ارزیابی تغییرات میزان آنزیم‌های آنتی اکسیدانسی و مالون‌دی‌آلدئید به عنوان محصول جانبی اکسیداسیون چربی در کبد تاس‌ماهی ایرانی در مواجهه با غلظت‌های تحت کشنده کلرید کادمیوم برای مدت ۱۴ روز می‌باشد. بر اساس نتایج فعالیت SOD و CAT افزایش معنی‌داری را در مواجهه با غلظت‌های مختلف کادمیوم تا روز ۷ام نشان داد و سپس فعالیت آن‌ها تا روز ۱۴ام در همه تیمارها کاهش یافت. در تیمارهای مورد بررسی میزان مالون‌دی‌آلدئید از روز اول تا انتهای دوره آزمایش افزایش یافت. میزان SOD، CAT و مالون‌دی‌آلدئید روند وابسته به دوزی تبعیت کرده و در تیمار ۸۰۰ میکروگرم در لیتر بالاتر از تیمار ۲۰۰ میکروگرم در لیتر بود. نتایج نشان داد که اندازه‌گیری آنزیم‌های آنتی اکسیدانسی و مالون‌دی‌آلدئید می‌تواند به عنوان شاخص مؤثری در پایش تغییرات سم‌شناسی محیطی باشد.

کلمات کلیدی: استرس اکسیداتیو، سوپراکسید دیسموتاز، کاتالاز، مالون‌دی‌آلدئید، تاس‌ماهی ایرانی.